

the state of the s

Plasmid Backbone

FIGURE 2A

FIGURE 2B

GTTAACTACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATA TGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTC CGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGA $\tt TGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCC$ ${\tt TCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAG}$ GCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCA $\tt CTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAG$ ${\tt ACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTG}$ ATTTACCCCGGTTGATAATCAGAAAAGCCCCAAAAACAGGAAGATTGTATAAGCAAATATTTAAATTGTAAACGTTAATA $\tt TTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTAT$ AAATCAAAAGAATAGCCCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTC ${\tt GAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTACGGTTCCTGGCCTTTTGCTCACATGTAATGTG}$ AGCGGCGCGCGAATTCCTGCAGGATTCGAGGGCCCCTGCAGGTCAATTCTACCGGGTAGGGGAGGCGCTTTTCCCAAGG ${\tt TCCACCGGTAGCGCCAACCGGCTCCGTTCTTTGGTGGCCCCTTCGCGCCACCTTCTACTCCTCCCCTAGTCAGGAAGTTC}$ ${\tt TGTCATCTCACCTTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCT}$ TGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGATG ${\tt ATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGT}$ ${\tt GGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTTCCCGATTCGCAGCGCATCGCCTTCTTATCGCCTTCTTGACGAGT}$ ${\tt TCTTCTGAGGGGATCGATCCGTCCTGTAAGTCTGCAGAAATTGATGATCTATTAAACAATAAAGATGTCCACTAAAATGG}$ $\tt GTGGGGGTGGGGTTAGATAAATGCCTGCTCTTTACTGAAGGCTCTTTACTATTGCTTATGATAATGTTTCATAG$ $\tt TTGGATATCATAATTTAAACAAGCAAAACCAAATTAAGGGCCAGCTCATTCCTCCCACTCATGATCTATAGATCTATAGA$ ${\tt TCTCTCGTGGGATCATTGTTTTTTCTCTTGATTCCCACTTTGTGGTTCTAAGTACTGTGGTTTCCAAATGTGTCAGTTTCA}$ TAGCCTGAAGAACGAGATCAGCAGCCTCTGTTCCACATACACTTCATTCTCAGTATTGTTTTGCCAAGTTCTAATTCCAT ${\tt CAGAAGCTGACTCTAGATCTGGATCCGGCCAGCTAGGCCGTCGACCTCGAGTGATCAGGTACCAAGGTCCTCGCTCTGTG}$ TATTACGGACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACA

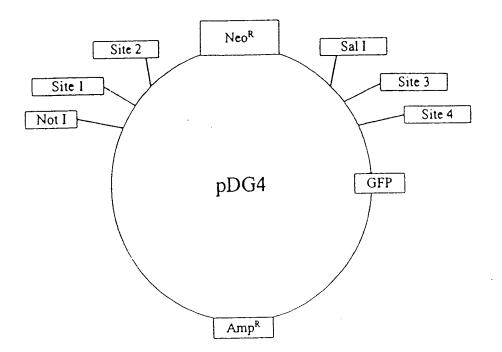


FIGURE 3A

FIGURE 3B

GTTTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGG CCCGCCTGGCTGACCGCCCAACGACCCCCCCCCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGA $\tt CTTTCCAATGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT$ AGCTGGTTTAGTGAACCGTCAGATCCGCTAGCGCTACCGGTCGCCACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGG AGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCGAGGTGAAGTTCGAGGGCGACAACCACTACCTGAGGACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTC CATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATA AAATGAATGCAATTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTC ${\tt TTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGATGCTGAAGATCAG}$ TCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTC GCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTA ${\tt TACCAAACGACGAGCGTGACACCACGA1GCCTGTAGCAATGGCAACGATGCGCAAACTATTAACTGGCGAACTACTT}$ ${\tt ACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCC}$ $\tt CGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAAT$ $\tt GTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACCTTCAAGAACTC$ TGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCCCAGTGGCGATAAGTCGTGTCTTACCG GGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTG GGAAACAGCTATGACCATGATTACGCCAAGCTACGTAATACGACTCACTAGGCGGCCGCGTTTAAACAATGTGCTCCTCTGCGCTTTAGCAGCCCCGCTGGCACTTGGCGCTACACAAGTGGCCTCTGGCCTCGCACACATTCCACATCCACCGGTAGCG ${\tt GCCATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACA}$ $\tt CTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCT$ ACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAA GAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGATGATCTCGTCGTGAC $\tt CTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTTATCGCCTTCTTGACGAGTTCTTCTGAGGGGA$ ${\tt TCGATCCGTCCTGTAAGTCTGCAGAAATTGATGATCTATTAAACAATAAAGATGTCCACTAAAATTGGAAGTTTTTCCTGT}$ GGGATTAGATAAATGCCTGCTCTTTACTGAAGGCTCTTTACTATTGCTTTATGATAATGTTTCATAGTTGGATATCATAATTTAAACAAGCAAAACCAAATTAAGGGCCAGCTCATTCCTCCCACTCATGATCTATAGATCTATAGATCTCTCGTGGGAT CATTGTTTTTCTCTTGATTCCCACTTTGTGGTTCTAAGTACTGTGGTTTCCAAATGTGTCAGTTTCATAGCCTGAAGAAC GAGATCAGCAGCCTCTGTTCCACATACACTTCATTCTCAGTATTGTTTTGCCAAGTTCTAATTCCATCAGAAGCTGACTC ${\tt ACGACACGGACACGCAAATTAATTAAGGCCGGCCCGTACCCTCTAGTCAAGGCCTTAAGTGAGTCGTATTACGGACTGG}$ $\tt CCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCC$ AGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTCGC TTGGTAATAAAGCCCGCTTCGGCGGGCTTTTTTTT

FIGURE 3B (Continuted)

₹	*
٤	4
C	2
-	2
ζ	١
í	ī

		Sequence after digestion
Annealing	Sequence	
site		F. tatantontatttagettagettecaa 3'
	5, tgtgctcctcttggcttgcttccaa3	3' cycycacacacacacacacacacacacacacacacacac
•	3 acacyayyayaaaaa	5. craattettatetagettageceaa 3'
r	5' ctggttcttgtctggcttggcccaa 3'	3' tt 5'
7	3 · gaccaagaacagaccgaaccaaa	r. sationtofotototoaa3.
	5' ggtcctcgctctgtgtccgttgaa3'	a gyreceegeegegegeegegegege
٣	3' ccaggagcgagacacaggcaactt 3	
	r. +++andiatoctatatogtogaa 3'	5 tregograficoraracumonadas
4	5	3.
-	5	

				Sequence after digestion	_
Annealing		Sequence	- 		
site				t	
	5.00	AAtgrgctcctcttggcttgcttCCGC 3	 	AA Ttacacgaggagaaaccgaacgaagg 5	-
	2	רמרמכי	- u	3	M
~	ν.	AActggttcttgtctggcttggctcccc	n m	Ttgaccaagaacagaccgaaccggg 5	5,
1	· n	1 Cyaccaayaacayaaay	-	C ad	٠,
,	S	AAggtoctogototgtgtoogttGAGG1 3	- m	Trecaggagegagacaeaggeaae	5 '
1	3-	Trocaggagegagacacagges	- 0	A A	3.
4	30	AAtttgcgtgtcctgtgtcgtcGAGCI		Traaacgcacaggacacagcagc	- 2
r	- m 	Tradacycacaggacacag			

FIGURE 5

FIGURE 6

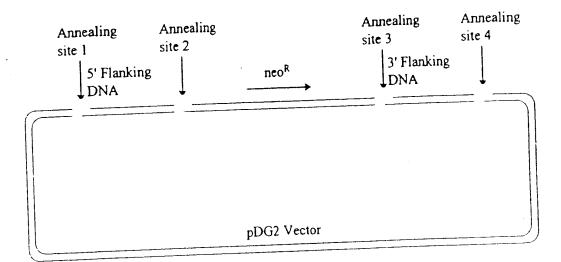
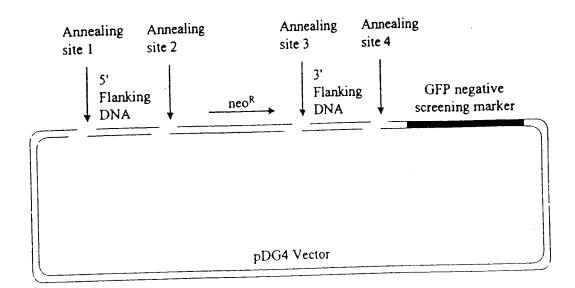


FIGURE 7



TTCCTGACAAGACTATGTCCACTCAGGAGCCCCAGAAGAGTCTTCTGGGTTCTCTCAACTCCAATGCCAC CTCTCACCTTGGACTGGCCACCAACCAGTCAGAGCCTTGGTGCCTGTATGTGTCCATCCCAGATGGCCTC TTCCTCAGCCTAGGGCTGGTGAGTCTGGTGGAGAATGTGCTGGTTGTGATAGCCATCACCAAAAACCGCA ACCTGCACTCGCCCATGTATTACTTCATCTGCTGCCTGGCCCTGTCTGACCTGATGGTAAGTGTCAGCAT CAGCTGGACAACCTCATTGACGTGCTCATCTGTGGCTCCATGGTGTCCAGTCTCTGCTTCCTGGGCATCA TTGCTATAGACCGCTACATCTCCATCTTCTATGCGCTGCGTTATCACAGCATCGTGACGCTGCCCAGAGC ACGACGGGCTGTCGTGGGCATCTGGATGGTCAGCATCGTCTCCAGCACCCTCTTTATCACCTACTACAAG CACACAGCCGTTCTGCTCTGCCTCGTCACTTTCTTTCTAGCCATGCTGGCACTCATGGCGATTCTGTATG CCCACATGTTCACGAGAGCGTGCCAGCACGTCCAGGGCATTGCCCAGCTCCACAAAAGGCGGCGGTCCAT CCGCCAAGGCTTCTGCCTCAAGGGTGCTGCCACCCTTACTATCCTTCTGGGGATTTTCTTCCTGTGCTGG GGCCCCTTCTTCCTGCATCTCTTGCTCATCGTCCTCTGCCCTCAGCACCCCACCTGCAGCTGCATCTTCA AGAACTTCAACCTCTTCCTCCTCCTCATCGTCCTCAGCTCCACTGTTGACCCCCCTCATCTATGCTTTCCG AGGGTGACAGTGATATCCAGTGGCCTGCATCTGTGAGACCACAGGTACTCATCCCTTCCTGATCTCCATT GCAAGGGTCAGACCACAGGCTCCTGAAGAGCTTCACCTCTCCCCACCTACAGGCAACTCCTGCTCAAGCC (SEQ ID NO: 19)

Targeting Vector (5' arm; 200 bp flanking neo insert):

CCGACAACAACATGAAGTGAATCAGAAGCTGGGGGCTGATACCACCTGGAGCTGCAG CCTCCACAGACCGCTTCCTACTTCCTGACAAGACTATGTCCACTCAGGAGCCCCAGAA GAGTCTTCTGGGTTCTCTCAACTCCAATGCCACCTCTCACCTTGGACTGGCCACCAACC AGTCAGAGCCTTGGTGTCTGTATGTG (SEQ ID NO: 20)

Targeting Vector (3' arm; 200 bp flanking neo insert):

GACTACTATCATCCTGCTGCTGGAGGTGGGCATCCTGGTGGCCAGAGTGGCTTTGGTG CAGCAGCTGGACAACCTCATTGACGTGCTCATCTGTGGCTCCATGGTGTCCAGTCTCT GCTTCCTGGGCATCATTGCTATAGACCGCTACATCTCCATCTTCTATGCGCTGCGTTAT CACAGCATCGTGACGCTGCCCAGAG (SEQ ID NO: 21)